# IBE: Articles

## Behavioral Monitoring of Trained Insects for Chemical Detection

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A portable, handheld volatile odor detector ("Wasp Hound") that utilizes a computer vision system and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid wasp, as the chemical sensor was created. Five wasps were placed in a test cartridge and placed inside the device. Wasps were either untrained or trained by associative learning to detect 3-octanone, a common fungal volatile chemical. The Wasp Hound sampled air from the headspace of corn samples prepared within the lab and, coupled with *Visual Cortex*, a software program developed using the LabView graphical programming language, monitored and analyzed wasp behavior. The Wasp Hound, with conditioned wasps, was able to detect 0.5 mg of 3-octanone within a 240 mL glass container filled with feed corn ($\approx 2.6 \times 10^{-5}$ mol/L). The Wasp Hound response to the control (corn alone) and a different chemical placed in the corn (0.5 mg of myrcene) was significantly different than the response to the 3-octanone. Wasp Hound results from untrained wasps were significantly different from trained wasps when comparing the responses to 3-octanone. The Wasp Hound may provide a unique method for monitoring grains, peanuts, and tree nuts for fungal growth associated with toxin production, as well as detecting chemicals associated with forensic investigations and plant/animal disease.

### Introduction

Traditional methods of volatile detection include utilizing human and canine olfaction, gas chromatography/mass spectroscopy (GC/MS), and the electronic nose. However, a new field is emerging around harnessing the sensing systems of nontraditional biological organisms where speed of detection and high sensitivity are necessary.

The U. S. Army Center for Environmental Health Research (USACEHR) has devised a method of using bluegill sunfish (*Lepomis macrochirus*) for monitoring a broad range of toxins in fresh water (*1*). The aquatic biomonitor uses mounted electrodes to monitor electric signals generated in the water by the movement of the fish. Ventilatory parameters, such as ventilatory rate, ventilatory depth, gill purge (cough) frequency, and whole-body movement are monitored and initiate an alarm when detected as abnormal. The system responds within an hour to most chemicals at toxic levels. This aquatic biomonitor is currently being implemented in a New York City reservoir.

Research by Apopo at the Sokoine University of Agriculture in Tanzania has led to the development of a successful regiment for training African Giant Pouched rats (*Cricetomys gambianus*) to nondestructively detect landmines and accurately detect Tuberculosis from sampled sputum (*2*). The rats have shown success in discriminating between positive and negative sputum samples without the need for expensive test equipment. Using rats to perform these tasks may have significant impact on TB control in low income countries. The rats are also capable of residual explosive scent tracing (REST) and direct detection of buried mines. The rats can be brought samples for identification or taken out and led through suspected mine fields.

Inscentinel Ltd. (Hertfordshire, UK) has successfully devised and marketed a system using honey bees (*Apis mellifera* [Hymenoptera: Linnaeus]) for trace vapor detection (*3*). Using specially designed hardware and image recognition software, Inscentinel can monitor the activity of honey bees inside their patented cassettes. The system's electronic output can notify a user of the presence of a single target odor.

Like the bees utilized by Inscentinel Ltd., trained *Microplitis croceipes* can be used to identify target odors (*4*−*6*). *M. croceipes* is a parasitic wasp (larval parasitoid) that has a highly sensitive olfactory system for finding food and hosts. The life cycle of *M. croceipes* relies on its well-developed ability to track chemical cues from plant canopies and hosts such as short, straight carbon chains, aromatic hydrocarbons, and complex multiring sesquiterpenes (*5*, *7*). Through associative learning, *M. croceipes* can link odors to host or food as their environment changes (*8*). These wasps can also be hand-trained in the laboratory to associatively learn to link odors to food and host within 5 min (*5*). *M. croceipes* respond to a trained odor with a behavioral response, which indicates a positive identification. Observable behaviors that indicate detection of the target odor include searching around the source of the odor and head-sticking into a hole emitting the odor.

In addition to the odors experienced naturally, *M. croceipes* are also capable of learning a wide spectrum of anthropogenic

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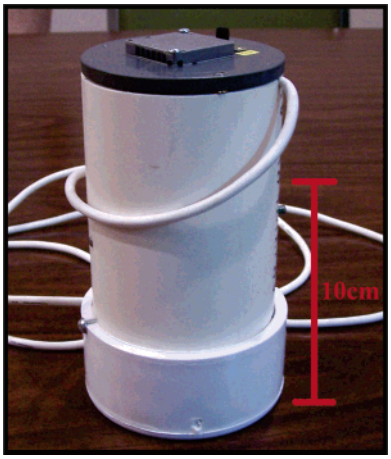



**Figure 1.** The Wasp Hound integrates a computer vision system into a portable handheld detector. The enclosure provides consistent lighting, cartridge placement, and air flow.

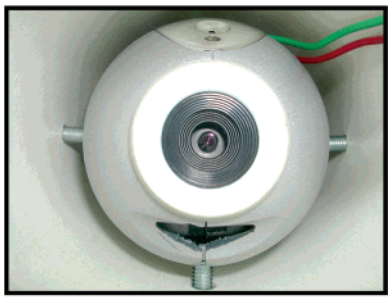
chemical compounds in association with both food and host at levels of $10^{-6}$ mol $L^{-1}$ (*9*), which is in the same order of sensitivity as canines (*10*). *M. croceipes* has been trained to a large array of chemicals associated with detection of food spoilage and toxins (*11*), plant disease (*9*), and forensics investigations (*12*). In one study, *M. croceipes* had a 10-fold higher sensitivity than a commercially available electronic nose (*9*). In addition, *M. croceipes* are cheap to reproduce and are easily and quickly trained, compared to canines, which can take 6 months and cost around $15,000 to train and require a dedicated handler and maintenance (vet bills, food, housing) (www.searchdogfoundation.org). Consequently, a sensing system utilizing the olfactory system of *M. croceipes* would be flexible, sensitive, portable, and potentially less expensive compared to canines.

The objective of this study was to investigate the effectiveness of a portable handheld instrument (Wasp Hound) utilizing *M. croceipes* as an olfactory sensor to detect volatile organic compounds. The Wasp Hound must be able to detect the search behavior of *M. croceipes*. The Wasp Hound was connected to a laptop PC and air samples were brought into the device. The target compound, 3-octanone, is a common odor associated with fungal growth, such as *Sclerotium rolfsii* (*13*), a fungal pathogen of several plant species. The device was also tested to determine how well it can discriminate between the target odor (3-octanone), a nontarget odor (myrcene), and a background odor (plain corn).

## Materials and Methods

**Handheld Design.** The Wasp Hound was developed as a handheld instrument for the detection of volatile compounds (Figure 1). The Wasp Hound consists of a ventilated area, a mounted camera, fixed light source, and test cartridge loading area. The device's air sampling method creates an odor gradient inside the device by slowly drawing outside air through the test cartridge at 4 mL/min. The mounted camera is used for observing insect behavior during testing. Images are transferred to the laptop PC and analyzed with a software program called *Visual Cortex* (*14*).

**Electrical.** The Wasp Hound contains electrical components for acquiring images of insect behavior, air sampling, and lighting the interior of the enclosure. All power and communication for the Wasp Hound is done through a USB (Universal Serial Bus) connection to a laptop PC. Images are acquired using a Logitech QuickCam with lighting provided



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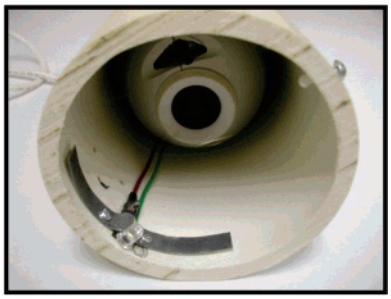

B

**Figure 2.** Mounting of camera and LED within Wasp Hound. (A) The Logitech QuickCam was suspended within the PVC body by three bolts 2.5 cm above the test cartridge top. (B) An LED was mounted within the Wasp Hound as the main source of illumination.

by a white 2300 mcd LED and a current-limiting 46 Ω resistor. Ventilation is provided by a flat unidirectional CPU fan whose speed is changeable between "Purge" (12 mL/min) and "Test" (4 mL/min) by using a single pole double throw (SPDT) switch and a 56 Ω current-limiting resistor.

**Enclosure.** The Wasp Hound enclosure keeps all components positioned statically and allows for consistent uniform lighting (Figure 2). Body and cap are made from PVC (poly(vinyl chloride)). The body is made of a 15.7 cm long piece of 7.6 cm schedule 40 PVC pipe. The bottom of the body was capped with a flat-bottomed PVC cap. The center of the inside of the cap was bored out to a depth of 0.3 cm and diameter of 4.0 cm. A 0.4 cm diameter through hole was made in the center of bore. Two custom-made clips are located on each side of the bore and are used to hold a test cartridge in place. The top of the body is capped with a flat, circular piece of 0.6 cm thick plastic. A 2.3 cm diameter hole was cut in the top to allow for mounting of the ventilation fan. Inside the body of the Wasp Hound, a custom-made bracket is mounted for holding the LED in place.

**Software.** Communication with the Wasp Hound was through the *Visual Cortex* interface (14). All image acquisition and analysis was performed using this software.

**Test Cartridge Design.** A cartridge was needed to adequately allow for sufficient movement of 5 female *M. croceipes* wasps but not so large as to diminish their timely responsiveness (Figure 3). Additionally the cartridge had to be well-ventilated and transparent so the interior could be monitored and illuminated.

The cartridge was composed of three parts. The body of the cartridge was part of a Millipore Aerosol Analysis Monitor. The top was a lid for a Millipore PetriSlide modified to fit the body and thoroughly perforated to allow for ventilation. A wire mesh disk was placed in the bottom of the body to prevent the wasp from escaping through the inlet.

**Insects.** *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) were used for this investigation. *M.* croceipes are larval

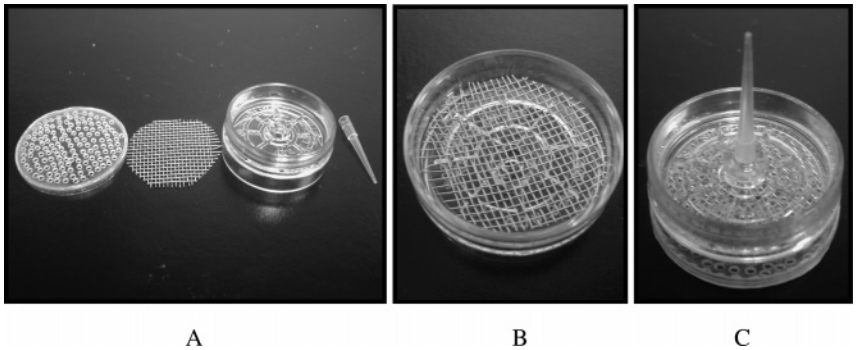


**Figure 3.** Test cartridge. (A) The test cartridge was composed of a top from a Millipore PetriSlide, a wire mesh disk, part of a Millipore aerosol analysis monitor, and a FinTip Pipet tip. (B) The mesh disk was placed in the body of the cartridge to prevent *M. croceipes* from escaping through the bottom. (C) The top fit onto the body and prevented *M. croceipes* from flying away while providing adequate ventilation, and the pipet tip was inserted into the bottom to direct air samples into the cartridge.

parasitoids of *Heliothis virescens* (tobacco budworm) and *Helicoverpa zea* (corn earworm). They are black nectar feeding wasps, approximately 10−12 mm in length and 2−3 mm in width, with a yellowish abdomen. The larval hosts used for rearing were *Heliothis zea* (Lepidoptera: Notuidae) and the procedures as described by Lewis and Burton (15) were followed. The breeding stock was provided with water and honey and kept in a Plexiglass cage (30 × 30 × 17 $cm^3$) at 28 °C, 50−70% RH, and a L16:D8 photocycle. Test specimens were females, 2 days old, given only water from time of emergence and no oviposition experience.

**Chemicals.** The chemicals used in this investigation were 3-octanone and myrcene. 3-Octanone ($C_8H_{16}O$, vapor pressure 1.5 mmHg) is a compound found in many fungal pathogens and myrcene ($C_{10}H_{16}$, vapor pressure 2.01 mmHg) is released by cotton plants when fed on by cotton bollworms (*7*).

Both chemicals were diluted using dichloromethane ($CH_2Cl_2$, vapor pressure 350 mmHg) as the solvent. When an aliquot of solution was allowed to evaporate, the dichloromethane evaporated quickly, leaving behind the less volatile solute (5).

**Training Procedure.** Five female *M. croceipes* were trained to associate 3-octanone with food. All training procedures were performed under a fume hood in the USDA-ARS Biological Control Laboratory in Tifton, GA. A fluorescent ring light was placed in the fume hood to lure escaped wasps.

An odor delivery stage was prepared for each group trained. First, a Whatman filter disk was impregnated with a 10 uL aliquot of 3-octanone/dichloromethane (1:16) solution and allowed to dry for 1 min. Each aliquot contained 1.0 mg of target chemical (3-octanone). Next, the filter disk was placed in a glass Petri dish (1.7 cm × 5.30 cm) that was in turn covered with a piece of aluminum foil (12 cm × 12 cm). The headspace within the covered dish was allowed to concentrate for 1 min, during which a piece of filter paper (2 mm × 2 mm) was placed in the center of the aluminum foil covering and saturated with 50% sucrose solution. Finally, a push pin was used to create 6 holes in a tight circular pattern around the sucrose water saturated filter paper.

Five *M. croceipes* females were captured and individually hand trained using the feeding regiment set forth by Olson et al. (*2*). The five wasps were captured from their rearing cage and placed in separate vials. Each wasp was removed individually and allowed to feed on the sucrose solution for 10 s. After feeding, each wasp was placed back in its vial. The process was repeated so each wasp was allowed to feed for three 10-s intervals with approximately 60 s between each feeding.

**Sample Preparation.** Three corn sample preparations were used for testing. Each preparation contained either corn and an odorant (test) or corn alone (control). Corn was utilized as a background odor potentially giving insight into the capabilities of utilizing *M. croceipes* for the detection of toxins present in large grain stores. The mouth of the jar for all samples were covered with a 12 cm × 12 cm piece of aluminum foil and shaken for 15 s. Test and control treatments were created by placing a Whatman filter disk in a 240 mL mason jar filled with 120 g of feed corn. The Whatman filter disk was impregnated with an aliquot of 3-octanone/dichloromethane (1:16) or myrcene/dichloromethane (1:16) solution on a glass dish and allowed to evaporate for 1 min. The resulting amount of 3-octanone and myrcene placed in the jar was 0.5 mg. If there is complete evaporation of the chemical within the jar of corn, the resulting concentrations are 2.6 × $10^{-5}$ and 2.5 × $10^{-5}$ mol/L for 3-octanone and myrcene, respectively. The disk was dropped onto the surface of the corn and pushed to the bottom before covering the jar. After shaking, samples were set aside to allow the headspace over the corn to build for 5 min. A total of 15 controls (corn only), 10 tests containing myrcene, and 10 tests containing 3-octanone were conducted.

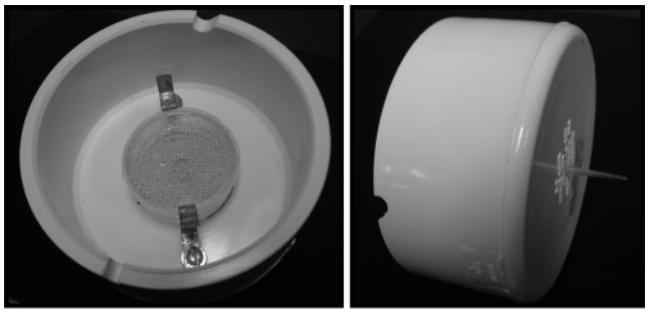


**Figure 4.** Test cartridge placement within the Wasp Hound. (A) During testing the test cartridge was placed within the cap and secured by two clips. (B) The pipet tip protruded through the bottom of the cap.

**Cartridge Preparation.** Five female *Microplitis croceipes* were confined in a cartridge during testing. Before using, each cartridge was thoroughly cleaned with soap and water. After drying, cleaning was continued by sweeping a 10 L/min air stream for approximately 15 s over all surfaces of the cartridge.

A Fintip plastic pipet tip was inserted into the bottom of the cartridge. The tip channeled all airflow directly from the corn sample to the inlet of the cartridge (Figure 4).

**Testing.** Data were collected using the Wasp Hound and *Visual Cortex* with both untrained and trained wasps presented with 3-octanone and myrcene odorants. Empty test cartridges (no wasps) were also tested to check for the uniformity of

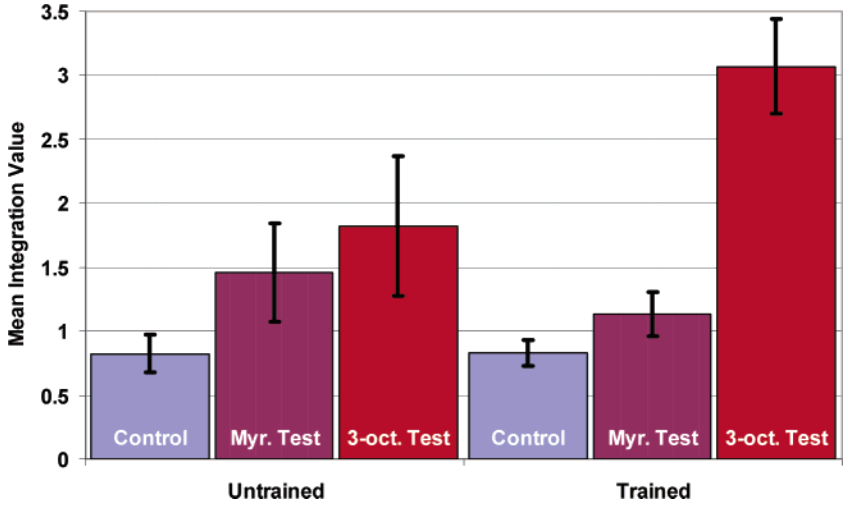


**Figure 5.** The mean responses for treatments per training.

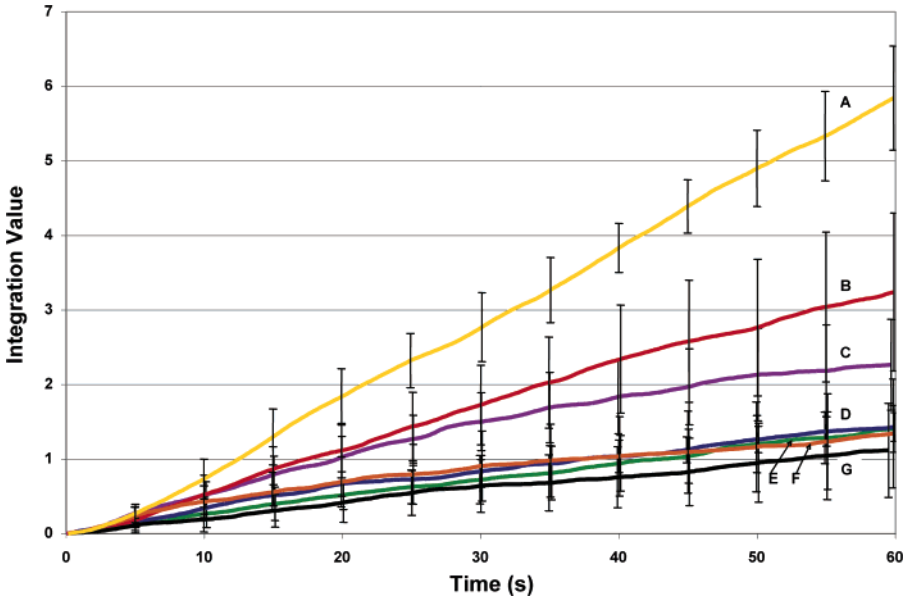


**Figure 6.** Means and 95% confidence intervals for treatments of trained (A, F, G) and untrained (B, C, D, E) *M. croceipes*. The mean response of *M. croceipes* trained to detect 3-octanone receiving 3-octanone test (A) treatments was measured as significantly different from untrained wasps receiving 3-octanone (B) and myrcene (C) test and control (D, E) treatments, trained wasps receiving myrcene test (F), and control (G) treatments after 25 s.

cartridges and lighting (blank treatment). The searching behavior of *M. croceipes* was observed under three conditions:

(1) Untrained wasps presented with control sample, then 3-octanone test sample

(2) Untrained wasps presented with control sample, then myrcene test sample

(3) Wasps trained to 3-octanone presented with control sample, then myrcene test sample, then 3-octanone test sample.

Images of the behavioral responses of untrained wasps when presented with either odor were collected, to determine if *M. croceipes* has a natural attraction to either 3-octanone or myrcene. Also during this time, 10 blank treatments were tested. A test cartridge with five trained wasps was placed into the W*asp Hound*. The Wasp Hound was then switched to the test setting and placed over the prepared sample. Pictures were captured at ~4 frames/s using the visual cortex software.

**Analysis.** All pictures collected were analyzed with *Visual Cortex*. For each set of pictures, a black 320 pixel × 240 pixel TIF image containing a centered 125 pixel diameter white circle was used as a mask. The region-of-interest (ROI) set by the mask corresponded to a 1.4 cm diameter circular region. A lower threshold of 70 was used for binary segmentation (pixel values $<70$ forced to 0 and $>70$ forced to 1). *Visual Cortex* then provided data describing the number of black pixels within the ROI. The number of pixels, the percent of total pixels, and the integration of the time variant percent of total pixels that were black within the ROI were calculated for each set of pictures. A higher percentage of black pixels in the ROI corresponded to a higher level of wasp searching behavior.

Initial analysis revealed large variations in background noise (black pixels) between the 10 blank treatments (empty cartridges). To remove the "noise", each treatment was calibrated by electronically subtracting the background noise before conducting the tests.

Microsoft's *Excel* was used to graph the integration values and their corresponding time stamps for all replications (untrained wasps: 10 controls, five myrcene tests, five 3-octanone tests; trained wasps: five controls, five myrcene tests, five

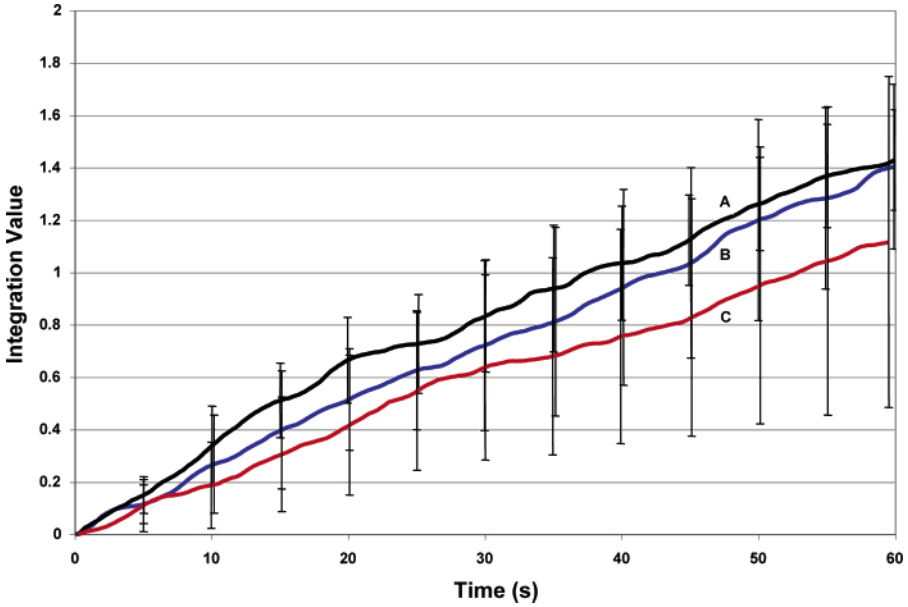


**Figure 7.** Means and 95% confidence intervals for no odor treatment responses for *M. croceipes* trained (C) and not trained (A and B) to 3-octanone.

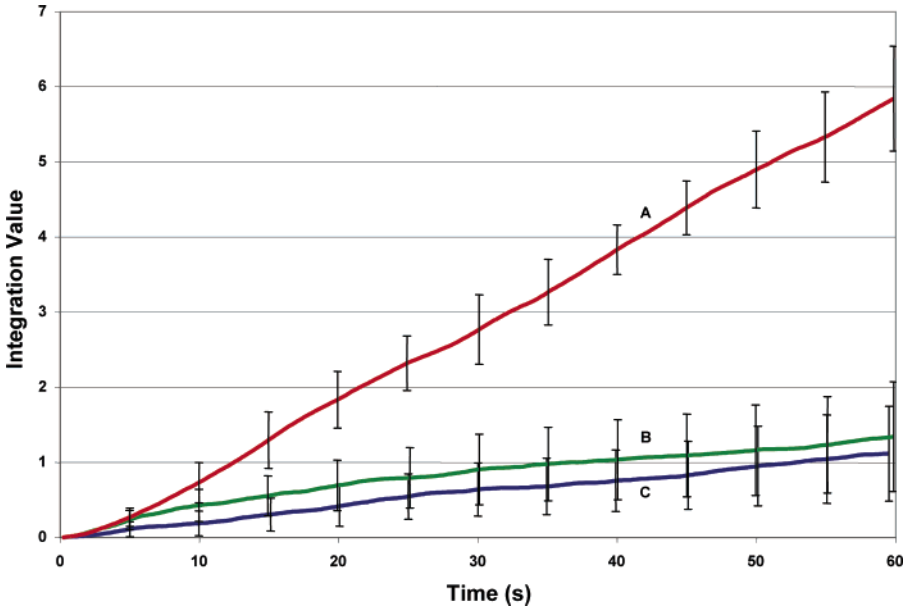


**Figure 8.** Means and 95% confidence intervals for 3-octanone (A), myrcene (B), and control (C) treatment responses of *M. croceipes* trained to detect 3-octanone.

3-octanone tests). The standard deviation was calculated for the integration values every 5 s (excluding zero). Confidence intervals were calculated using $\alpha = 0.05$ and $n = 5$.

An ANOVA statistical analysis of the data was performed using a general linear model (SAS). Observations were taken from the 15 groups (5 individuals per group) of *M. croceipes* divided unevenly into two levels of training (10 groups untrained, 5 groups trained). The untrained groups were further divided into two sets. One untrained set received control and myrcene treatments. The other untrained set received control and 3-octanone treatments. The five groups of trained *M. croceipes* received control, myrcene, and 3-octanone test treatments. Each treatment was replicated five times. The 10 blank treatment replications were analyzed to determine if each was statistically the same. The remaining 35 replications (15 controls, 10 myrcene tests, 10 3-octanone tests) were analyzed by training and by treatment to determine if either factor had a significant effect on the mean response. A total of 45 tests were conducted and analyzed (Table 1). Analysis between treatments and training were also examined at 5 s time intervals from 0 to 60 s.

**Table 1. Treatment Layout**[a]

| | treatment | | | | |
|---|---|---|---|---|---|
| | blank | control | myrcene | 3-octanone | Σ |
| untrained | 10 reps | 10 reps | 5 reps | 5 reps | 30 reps |
| trained | 0 reps | 5 reps | 5 reps | 5 reps | 15 reps |
| Σ | 10 reps | 15 reps | 10 reps | 10 reps | 45 reps |

[a] Blank (no odor, no wasps) and control (no odor, 5 wasps) treatments were replicated 10 times each using untrained *M. croceipe*. Myrcene (myrcene, 5 wasps) and 3-octanone test treatments (3-octanone, 5 wasps) were each replicated five times using untrained *M. croceipes*. Control, myrcene, and 3-octanone treatments were replicated five times each using *M. croceipes* trained to detect 3-octanone.

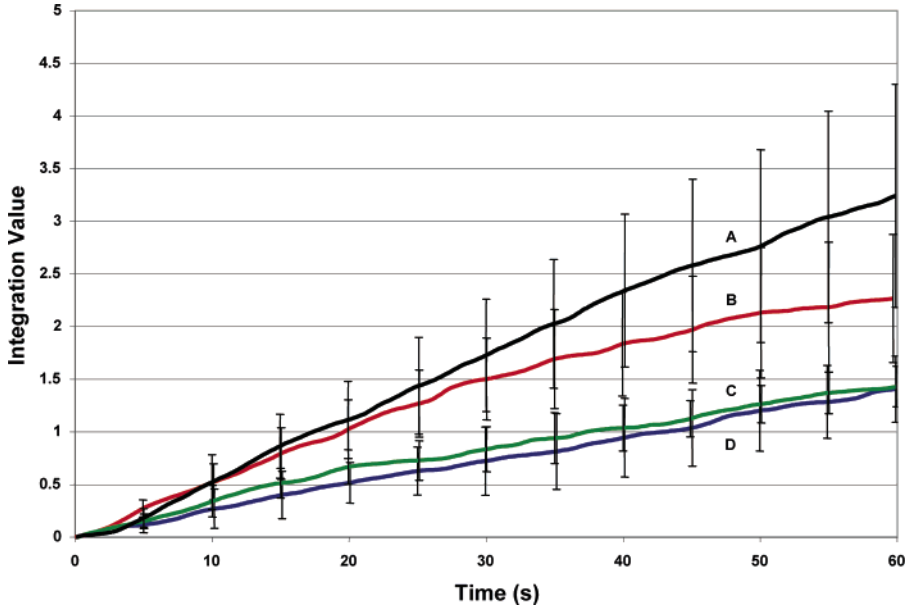


**Figure 9.** Means and 95% confidence intervals for 3-octanone (A), myrcene (B), and control (C and D) treatment responses of untrained *M. croceipes*.

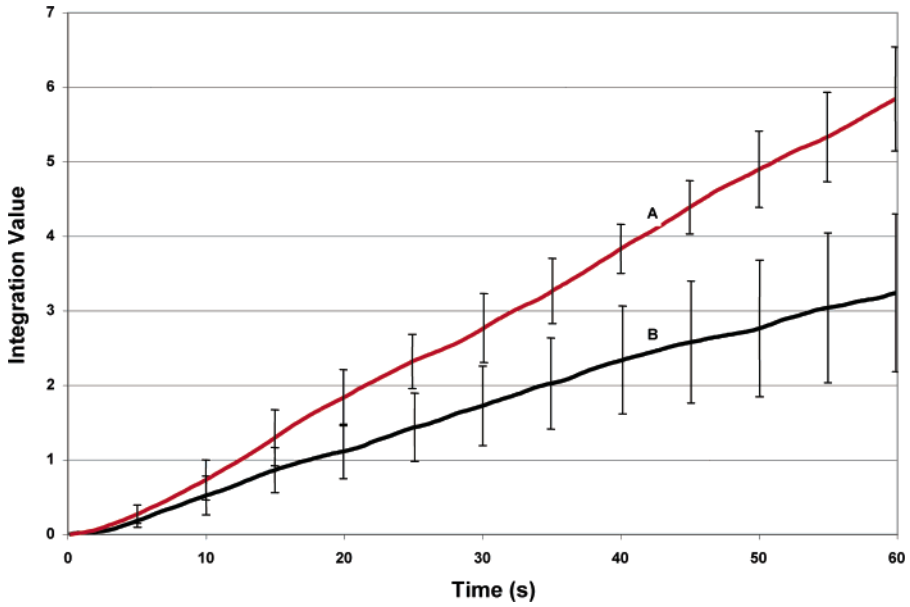


**Figure 10.** Means and 95% confidence intervals for 3-octanone treatment responses for trained (A) and untrained (B) *M. croceipes*.

## Results and Discussion

**Effects of Treatment and Training on Response.** The control and test treatments were analyzed to determine the effects of treatment and training on the mean response (average integration values over 60 s test period). Figure 5 shows the mean responses exhibited by untrained and trained *M. croceipes* grouped by training. The errors bars were calculated using $n = 5$ and $\alpha = 0.05$. The response of the *M. croceipes* groups over the 60 s test period can be seen in Figure 6. The response of *M. croceipes* trained to detect 3-octanone in the presence of the target odor was significantly different from all other treatment/training pairs after 25 s.

**Comparing Response of *M. croceipes* Groups Receiving Control Treatment.** Fifteen groups of *M. croceipes* received control treatments. Five groups received prior training to 3-octanone and 10 did not. The behavioral response of *M. croceipes* receiving the control treatment was not significantly different within or across training ($df = 2$, $n = 180$, $P < 0.80$). The response of the untrained wasps was not significantly different from each other or the mean response of *M. croceipes* trained to detect 3-octanone and receiving the same treatment (Figure 7). Similar results were shown by Utley et al. (*14*). This suggests that any cohort or day differences that existed did not influence or bias the amount of time *M. croceipes* spent within the ROI.

**Effects of Treatment on Response of *M. croceipes* Trained to 3-Octanone.** Five groups of *M. croceipes* trained to detect 3-octanone received control, myrcene, and 3-octanone treatments, in order. The behavioral response of *M. croceipes* trained to detect 3-octanone was significantly different across treatments ($df = 2$, $n = 180$, $P < 0.0001$) (Figure 8).

The mean response of the trained wasps exposed to 3-octanone (3.07) was significantly larger than that of trained wasps exposed to myrcene (1.13) or corn alone (0.83). No significant difference existed between the mean response of myrcene and control treatments. Trained *M. croceipes* showed interest in the myrcene, but the amount of searching elicited by the 3-octanone

was significantly greater than both the myrcene and control treatments within 15 s of exposure to the test treatment.

**Effects of Treatment on Response of Untrained M. croceipes.** Ten groups of untrained *M. croceipes* received control and test treatments, in order. Five groups were presented with 3-octanone during their test treatment, and five groups were presented with myrcene. The behavioral response of untrained *M. croceipes* was significantly different across treatments (df $= 3$, $n = 240$, $P = 0.0033$) (Figure 9).

The mean responses of untrained wasps exposed to 3-octanone (1.82) or myrcene (1.45) were not significantly different, but they were both significantly different from the mean responses of both control treatments (0.87 and 0.78). The control treatment responses were not significantly different. Again, the groups were not biased in the amount of time spent searching within the ROI but did show interest in the odorants without prior training or exposure.

Ten groups of wasps were exposed to 3-octanone. Five groups had received prior training to 3-octanone and five groups were untrained. The behavioral response of *M. croceipes* exposed to 3-octanone was significantly different across training (trained vs untrained) (df $= 1$, $n = 120$, $P = 0.01$) (Figure 10).

*M. croceipes* trained to detect 3-octanone had a significantly higher mean response (3.07) when presented with 3-octanone than did untrained wasps (1.82) receiving similar treatment. Untrained *M. croceipes* exhibited some searching behavior toward 3-octanone, but trained wasps exhibited significantly more searching.

## Conclusions

A portable handheld volatile chemical detector that interfaces with a laptop computer was constructed and tested. A computer vision system with image analysis software (*Visual Cortex*) successfully and objectively quantified the searching behavior of five trained female *M. croceipes* parasitoid wasps. The portable detector and image analysis software was able to distinguish between the strong searching behavior exhibited by trained *M. croceipes* when presented with 3-octanone within a background of corn and the random behavior observed during the control treatments. Training was shown to significantly affect the wasp response to target and nontarget odors. Although untrained wasps showed a higher response to the 3-octanone and myrcene than the control (corn only), when trained, their response to the target odor increased and for the nontarget odor decreased. Such a system has the potential to be utilized for the detection of target chemical odors within an environment containing a masking background. The handheld system constructed during this study quickly detected the presence of 3-octanone ($2.6 \times 10^{-5}$ mol/L) in a background of corn within 25 s.

The Wasp Hound could be used in several application areas. This study evaluated a potential use for detecting odors in stored grains that indicate development of fungi and/or toxins. Current detection of aflatoxin in peanut and corn relies on analyzing subsamples of the stored product. Using headspace of the stored commodities would provide a sample more representative of the entire stored product and could detect fungi and toxins that are missed with subsampling. Forensic investigations where the search for gravesites, illegal drugs, and accelerants used in arson could use a Wasp Hound in cases where canines are not available. Further studies are planned to illustrate the utility of the Wasp Hound in these situations.

**Note Added after ASAP Publication.** The author affiliations were incomplete in the version published ASAP September 29, 2005; the corrected version was published November 11, 2005.

## References and Notes

(1) Van der Schalie, W. H.; Shedd, T. R.; Widder, M. W.; Brennan, L. M. Response characteristics of an aquatic biomonitor used for rapid toxicity detection. *J. Appl. Toxicol.* **2004**, *24*, 387−394.

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